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Aromatase Inhibitors for Assisted Reproduction

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This chapter discusses the potential role of the new group of medications called ''aromatase inhibitors'' in assisted reproduction. In the past few years, aromatase inhibitors have emerged as promising agents for infertility treatment, particularly ovarian stimulation. We reported the first series in the literature of the successful application of an aromatase inhibitor for induction of ovulation in women with polycystic ovarian syndrome (PCOS) (1) and ovarian stimulation in women with ovulatory infertility, for example, unexplained infertility (2). Following this report, we showed the success of aromatase inhibition for controlled ovarian hyperstimulation (COH) (3–5), whether as a single- or multiple-dose administration (6), and suggested the potential application in various infertility treatments including assisted reproduction (7–13), with data on safety of the pregnancy outcome (14).

Since these first reports, more than a hundred clinical trials have been presented in international meetings, confirming our original findings, by investigators from all over the globe. Many peer-reviewed manuscripts (15–33) have already confirmed the success of aromatase inhibitors in various infertility applications. Currently, several multicenter clinical trials are running testing the clinical efficacy of aromatase inhibitors for infertility treatment including ovarian stimulation. In this chapter, we will discuss the potential role of aromatase inhibitors for assisted reproduction, presenting the theoretical benefits and the available evidence.

AROMATASE INHIBITORS

Blocking estrogen production by inhibiting the enzyme catalyzing its synthesis from androgens (aromatase enzyme) is a treatment modality that has been in clinical application for more than half a century since the development of the first generations of aromatase inhibitors including aminoglutethimide. However, the successful applications of aromatase inhibitors in managing estrogen-dependant disorders, particularly malignancies such as breast cancer, have not achieved significant success and popularity until recently. This was due to several problems encountered with the clinical use of the early generations of the aromatase inhibitors. Those problems could be overcome to a great extent by the development of the third generation of aromatase inhibitors. Table 24.1 summarizes the different generations of the aromatase inhibitors, while Boxes 24.1 and 24.2 summarize the significant problems associated with the early generations of the aromatase inhibitors and the advantages of the third-generation aromatase inhibitors, respectively.

The Aromatase Enzyme

Aromatase or ''estrogen synthase'' is the enzyme that catalyzes the rate-limiting step in estrogen synthesis, that is, the conversion of androgens (androstenedione and testosterone) into estrogens (estrone and estradiol, respectively). Many tissues express aromatase activity, in particular the ovaries, brain, adipose tissue, muscle, liver, breast tissue, and malignant tumors, for example, breast tumors. During the reproductive age (premenopausal women), the main source of circulating estrogens is the ovaries, while most of the circulating estrogen comes from the adipose tissue in women after menopause (34).

Aromatase Inhibitors

Being a terminal step in the cascade of steroidogenesis, aromatase is a good target for selective inhibition. A large number of aromatase inhibitors have been developed over the past five decades with the third-generation aromatase inhibitors licensed for suppressing estrogen synthesis in postmenopausal women with breast cancer in the past decade. The third-generation aromatase inhibitors were developed after the clinical failure of the earlier generations of aromatase inhibitors, as explained above in Boxes 24.1 and 24.2 (34, 35).

HYPOTHETICAL ROLE OF AROMATASE INHIBITORS FOR ASSISTED REPRODUCTION

We postulated that aromatase inhibitors can play a major role in the practice of assisted reproduction, both as ovarian stimulation agents as well as adjuvant agents that can enhance the outcome of assisted reproduction. Aromatase inhibitors can improve sensitivity to FSH and reduce complications such as severe ovarian hyperstimulation while lowering the overall cost of assisted reproduction. This section of the chapter is a hypothetical discussion of the underlying scientific justification supporting the application of aromatase inhibitors for assisted reproduction.

Mechanisms of Aromatase Inhibition for Ovarian Stimulation

Several years have now passed since the first report of the success of aromatase inhibition for ovarian stimulation. However, the underlying mechanisms behind the success of aromatase

Table 24.1: Different Generations of Aromatase Inhibitors

Generation	Nonsteroidal aromatase inhibitors	Steroidal aromatase inhibitors (sometimes called suicidal inhibitors of the aromatase enzyme)
	Work by temporary (reversible) inactivation of the aromatase enzyme	Work by permanent (irreversible) inactivation of the aromatase enzyme
First generation	Aminoglutethimide (Cytadren®)	NA
Second generation	Rogletimide	Formestane
	Fadrozole	
Third generation	Letrozole (Femara®, 2.5 mg/tablet)	Exemestane (Aromasin®, 25 mg/tablet)
	Anastrozole (Arimidex®, 1 mg/tablet)	
	Vorozole	

Box 24.1: Problems Associated with Early Generations Aromatase Inhibitors

PHARMACODYNAMIC ADVANTAGES:

1 Low potency in inhibiting the aromatase enzyme, particularly in premenopausal women (very low potency)
2 Lack of specificity in inhibiting the aromatase enzyme with significant inhibition of other steroidogenesis enzymes, leading to medical adrenalectomy

PHARMACOKINETIC ADVANTAGES:

1 Not all members are available orally (some require parenteral administration)
2 Variable bioavailability after oral administration
3 Variable half-life that changes with the period of administration due to induction of its metabolism

CLINICAL ADVANTAGES:

1 Poorly tolerated on daily administration, with more than a third of patients discontinuing treatment due to adverse effects
2 Significant side effects related to both the aromatase inhibitors, for example, drowsiness, morbilliform skin rash, nausea and anorexia, and dizziness and side effects secondary to the steroids used for replacement therapy, for example, glucocorticoids
3 Interaction with alcohol with significant potentiation of its action
4 Significant interactions with other medications, for example, coumarin and warfarin
5 Need for replacement therapy due to medical adrenalectomy, for example, glucocorticoid and mineralocorticoid replacement
6 Long-term possible carcinogenesis (at least in animals)

inhibition for ovarian stimulation have not been completely elucidated. We believe that several mechanisms, both central (at the level of the brain) and peripheral (at the level of the ovaries and the uterus), function together, with one or more mechanisms more important than the others in certain infertile subgroups.

Central Mechanisms

Centrally, blocking estrogen synthesis in the brain as well as lowering circulating estrogens by reducing whole-body estrogen synthesis would release the hypothalamus and/or pituitary from the estrogen-negative feedback on the production and release of gonadotropins (without depletion of estrogen receptors as occurs with antiestrogens, e.g., clomiphene citrate). The resultant increase in gonadotropin secretion will stimulate the growth of the ovarian follicles. Withdrawal of estrogen centrally also increases activins, which are produced by a wide variety of tissues including the pituitary gland (36) and will stimulate synthesis of FSH by a direct action on the gonadotropes (37).

Peripheral Mechanisms

Peripherally, aromatase inhibition may increase ovarian follicular sensitivity to FSH stimulation. This could result from temporary accumulation of intraovarian androgens since conversion of those androgen substrates to estrogens is blocked by inhibition of the aromatase enzyme. This assumption is based on data supporting a stimulatory role for androgens in early follicular growth in primates (38) mediated directly through testosterone augmentation of follicular FSH receptor expression (39, 40) and indirectly through androgen stimulation of

Box 24.2: Advantages of Third-Generation Aromatase Inhibitors

PHARMACODYNAMIC ADVANTAGES:

1 Extreme potency in inhibiting the aromatase enzyme (up to thousand times potency of the first-generation aminoglutethimide)
2 Very specific in inhibiting the aromatase enzyme without significant inhibition of the other steroidogenesis enzymes. This is true even at high doses
3 Absence of estrogen receptor depletion

PHARMACOKINETIC ADVANTAGES:

1 Orally administered (other routes of administration are also possible, e.g., vaginal and rectal)
2 Almost 100 percent bioavailability after oral administration
3 Rapid clearance from the body due to short half-life (approximately eight hours for the Aromasin® to approximately forty-five hours for the Femara® and Arimidex®)
4 Absence of tissue accumulation of the medications or any of their metabolites
5 No significant active metabolites

CLINICAL ADVANTAGES:

1 Well tolerated on daily administration for up to several years (in postmenopausal women with breast cancer) with few adverse effects
2 Few mild side effects
3 Very safe without significant contraindications
4 Absence of significant interactions with other medications
5 Very wide safety margin (toxic dose is several thousand times higher than recommended efficacious therapeutic dose)
6 Relatively inexpensive

insulin-like growth factor I (IGF-I), which may synergize with FSH to promote folliculogenesis (41, 42).

Reducing the Detrimental Effects of High Estrogen Levels Associated with COH

In women undergoing COH for assisted reproduction, supraphysiological levels of estrogen are inevitably attained due to the growth of multiple mature ovarian follicles. Whether such very high estrogen levels have detrimental effects on the outcome of assisted reproduction is still a matter of debate (10). Several explanations for the controversy include the different methodologies applied for assessing estrogen production during COH and the probability that different infertility subgroups are more vulnerable than others to the supraphysiological levels of estrogen (4, 44). A complete discussion of this issue is not within the scope of this chapter but the reader is referred to our recent review of the issue (10). Box 24.3 summarizes a list of potential mechanisms that have been postulated for detrimental effects of supraphysiological levels of estrogen on the outcome of infertility treatment including assisted reproduction (10).

When an aromatase inhibitor is applied during COH, estrogen production per growing ovarian follicle has been found to be significantly lower than when aromatase inhibitors are not used (about 40–60 percent less) (3–5). This makes sense as estrogen synthesis is significantly reduced by inhibiting the aromatase enzyme. We believe that the milder elevation in the levels of estrogen associated with aromatase inhibitor use during COH might improve the treatment outcome by reducing possible detrimental effects of supraphysiological estrogen levels that would be attained without aromatase inhibitors. This might be especially important in subgroups of patients who are probably more vulnerable to the high estrogen levels, for example, PCOS and women with endometriosis-associated infertility and in women with breast cancer (Oktay references) (22, 50, 51).

Reducing the Risk of Severe Ovarian Hyperstimulation Syndrome

Although there is a lack of consensus on the role of high estrogen levels in the pathophysiology and development of severe ovarian hyperstimulation syndrome (45), lowering the supraphysiological levels of estrogen by aromatase inhibitors seems to be a potential method of preventing or at least ameliorating the severity of severe ovarian hyperstimulation syndrome. Further investigation of this speculation is required.

In Vitro Maturation

The use of aromatase inhibitors for in vitro maturation is an exciting application that can involve a brief aromatase inhibitor–induced rise in endogenous gonadotropin secretion leading to multiple ovarian follicles, followed by retrieval of immature oocytes. Currently, there are no available data for such an application.

Box 24.3: Possible Detrimental Effects of the Supraphysiological Estrogen Levels Attained during Ovarian Stimulation and Assisted Reproduction

STRONG EVIDENCE:
Effect on the endometrium and implantation

1 Defective steroid hormones receptor development in particular estrogen, progesterone, and androgen receptors
2 Dyssynchronization of the stromal/epithelial endometrial development
3 Dyssynchronization between implantation window (advanced endometrial development while delayed in vitro development of the transferred embryos)
4 Abnormal temporal expression of the endometrial pinopodes (temporarily distant from the embryo transfer time)
5 Impaired endometrial blood flow (both subendometrial and possibly uterine blood flow)
6 Abnormal expression of the endometrial integrins and other adhesion molecules

REASONABLY STRONG EVIDENCE:
Effect on the developing gametes and embryos

1 Effect on chromosomal and cytogenetic integrity of the developing oocyte
2 Defective mitochondrial function in the oocyte
3 Effect on the sperm causing possible premature acrosome reaction and deactivation
4 Effect on the developing embryo and blastocyst hatching

LESS STRONG EVIDENCE:

1 Effect on the ovaries and pituitary (defective corpus luteum function and luteal phase) due to defective LH secretion, LH surge, and LH tonic pulse release
2 Other probable targets:
   a Abnormal leptin production
   b Excessive activation of the coagulation system
   c Defective development of placenta

Benefits in Particular Patient Groups

As mentioned earlier, both lowering supraphysiological levels of estrogen during COH and improving response to COH by enhancing endogenous gonadotropin production and increasing the ovarian follicular sensitivity to gonadotropin stimulation could be of benefit in particular groups of patients, for example, poor responders, endometriosis-associated infertility, PCOS, and survivors of estrogen-dependant malignancies, for example, breast cancer.

Poor Responders

As discussed in the sections of the mechanism of ovarian stimulation by aromatase inhibitors, administering aromatase inhibitors is expected to increase endogenous gonadotropin production and increase the sensitivity of the ovarian follicles to FSH stimulation according to the central and peripheral hypotheses, respectively. This would obviously be expected to enhance the response to COH in poor responders with accumulating evidence supporting this idea (3, 20). However, one should be cautious here as a good proportion of women with poor response to gonadotropins have depletion of the ovarian follicular reserve and early ovarian failure. Such a group of poor responders would not be expected to respond to any modality of COH, and their best chance for pregnancy lies in donor oocyte-assisted reproduction.

Endometriosis

The expression of the aromatase enzyme in endometriotic tissues and the significant role played by locally produced estrogen in endometriosis progression (4, 46, 47) suggests a benefit of aromatase inhibitors in endometriosis-related infertility. The inhibition of local estrogen production in endometrial implants and the lower peripheral estrogen levels associated with the use of aromatase inhibition for ovarian stimulation are expected to possibly protect against progression of endometriosis and may improve the outcome of assisted reproduction in this group of women. However, this idea still awaits confirmation by clinical trials.

Polycystic Ovarian Syndrome

Women with PCOS are at high risk of complications during COH and assisted reproduction, particularly severe ovarian hyperstimulation syndrome. As discussed earlier, those women could benefit from lowering estrogen levels by aromatase inhibition and possibly reduce the risk of severe OHSS.

Interestingly, aromatase inhibition can play a role at the level of the endometrium in those patients. Estrogen has been shown to decrease the level of its own receptor by stimulating ubiquitination of estrogen receptors. This results in rapid degradation of the receptors. In the absence of estrogen,

ubiquitination is decreased allowing upregulation of the estrogen receptors and increasing sensitivity to subsequent estrogen administration (48). This could increase endometrial response to estrogen, resulting in more rapid proliferation of endometrial epithelium and stroma and improved blood flow to the uterus and endometrium (49). As a result, normal endometrial development should occur despite the observed lower estrogen concentrations in aromatase inhibitor–treated cycle.

Survivors of Estrogen-Dependant Malignancies

Estrogen-sensitive cancers such as breast cancer can affect women in the reproductive age-group. Those women usually suffer from ovarian failure following chemotherapy particularly with alkylating agents. With the recent success of different fertility preservation options such as oocyte cryopreservation, some women may opt to freeze oocytes or embryos for later use by themselves or a gestational carrier. Oktay et al. reported the success of aromatase inhibition for COH in women undergoing assisted reproduction before receiving cancer treatment. Following COH, patients were followed for almost two years, during which the cancer recurrence rate was similar for patients who received aromatase inhibitor COH and those who had no ovarian stimulation (control patients) (50, 51).

AVAILABLE EVIDENCE FOR POTENTIAL ROLE OF AROMATASE INHIBITORS FOR ASSISTED REPRODUCTION

Evidence is accumulating to confirm the success of aromatase inhibitors as adjuvant agents to enhance the outcome of assisted reproduction. However, larger clinical trials are still needed to confirm and quantify the nature and extent of the benefits from adding aromatase inhibitors to COH stimulation protocols.

Reducing the Dose of Gonadotropins Required for COH and Improving Response in Poor Responders

A significant reduction in the gonadotropin dose required was found (45–55 percent reduction) when the aromatase inhibitor, letrozole, was added to gonadotropin COH treatment (3–5). Because gonadotropin injections constitute a significant part of the cost of infertility treatment, especially during assisted reproduction, we believe that aromatase inhibitors will markedly reduce the cost of infertility treatment by decreasing the gonadotropin dose required for optimum ovarian stimulation. This could make assisted reproductive technology available to a larger group of infertile couples. Moreover, such reduction in the gonadotropin dose observed by combining letrozole with gonadotropins encouraged us to explore the value of aromatase inhibition in improving ovarian response to gonadotropins in poor responders.

In a randomized, controlled study that included poor responders undergoing COH for assisted reproduction, Goswami et al. (20) compared letrozole plus gonadotropin protocol with a standard GnRH agonist and gonadotropin protocol. The study was a small pilot study that included thirty-eight patients. The authors found that adding the aromatase inhibitor, letrozole, to a small dose of gonadotropin (150 IU; two injections of 75 IU on cycle days 3 and 8) resulted in a similar number of oocytes retrieved, embryos transferred, and pregnancy rate as observed in the women on the standard protocol. Interestingly, the group that received the standard protocol had a mean ($\pm$SEM) total gonadotropins dose of 2865 $\pm$ 228 IU, that is, almost twenty times the total amount of gonadotropins received by the letrozole group.

In a more recent and larger study, Garcia-Velasco et al. (21) used the aromatase inhibitor, letrozole, as an adjuvant to gonadotropins in 147 poor responder patients undergoing COH for assisted reproduction. The patients had at least one previous assisted reproduction cycle that was canceled due to poor response to COH. The study was prospective but not randomized. The women were divided into a control group of seventy-six patients treated with high-dose gonadotropins in a GnRH-antagonist regimen. The experimental group included seventy-one patients who received the aromatase inhibitor, letrozole, at a dose of 2.5 mg plus gonadotropins for the first five days of stimulation followed by the same gonadotropin/antagonist regimen. The authors found women who received letrozole had higher numbers of oocytes retrieved with a higher implantation rate despite receiving the same doses of gonadotropins as the control group. Interestingly, both testosterone and androstenedione concentrations were significantly increased in the follicular fluid in the experimental group compared to the control group. These findings are consistent with our peripheral hypothesis that aromatase inhibition, by blocking androgen to estrogen conversion, increases intraovarian androgens and follicular FSH receptor expression and sensitivity to FSH administration.

Most recently, Verpoest et al. (33) in a pilot study randomized patients to receive letrozole (group A; $n = 10$), versus no letrozole (group B; $n = 10$) in an ovarian stimulation protocol with recombinant FSH 150 IU/day starting on day 2 of the cycle and gonadotropin-releasing hormone antagonist 0.25 mg/day starting on day 6 of the cycle. The authors found significantly higher LH concentrations in group A versus group B during letrozole administration. Group A (the letrozole group) was also associated with lower estradiol concentrations but higher serum FSH, testosterone, and androstenedione concentrations compared to group B, throughout the follicular phase. However, the differences were not statistically significant. Median endometrial thickness was significantly higher in group A (letrozole) on the day of human chorionic gonadotropin administration. Pregnancies were higher in the letrozole group. However, the small sample size did not allow the difference to reach statistical significance. The authors concluded that their pilot study supported the idea that aromatase inhibitors can contribute to normal implantation and follicular response, without having negative antiestrogenic effects (33).

Safety Concerns about Aromatase Inhibitors for Ovarian Stimulation

With the short period of clinical experience with aromatase inhibitors for infertility treatment, caution needs to be applied and proper patient consent is necessary for full approval of the ovarian stimulation indication to be attained.

Side Effects of Aromatase Inhibitors

Most of the data about side effects associated with clinical use of aromatase inhibitors come from clinical trials involving postmenopausal women with breast cancer. In this group of patients, third-generation aromatase inhibitors were generally well tolerated. The main side effects are hot flushes and gastrointestinal events (nausea and vomiting) and leg cramps. In

those trials, very few patients withdrew from first- or second-line comparative phase III trials because of drug-related adverse events with aromatase inhibitors (50–52). Obviously, these adverse effects were observed in older women with advanced breast cancer who received aromatase inhibitors daily over a long period of time for several months up to few years. Obviously, fewer adverse effects are expected in women who would receive aromatase inhibitors while undergoing assisted reproduction. Those women are usually healthier and younger and would receive the aromatase inhibitors for a short course of few days. In our clinical experience with the use of aromatase inhibitors for ovarian stimulation and in patients undergoing assisted reproduction, we have observed few side effects such as hot flushes and PMS-type symptoms. Interestingly, most of the patients who had a history of treatment with clomiphene citrate found treatment with an aromatase inhibitor to be better tolerated with fewer side effects compared to the clomiphene citrate (53). However, so far, there are no clinical trials that compared the adverse effects associated with the use of aromatase inhibitors for ovarian stimulation with other ovarian stimulation agents.

Low Estrogen Levels Associated with Aromatase Inhibitor Treatment

As expected, aromatase inhibition results in estrogen levels that are significantly lower when compared to serum estrogen levels at midcycle seen with treatment with other ovarian stimulation agents such as gonadotropins or clomiphene citrate. Midcycle estradiol levels per mature follicle were around half that found without aromatase inhibitors treatment (3–5). The question whether low or very low intrafollicular estrogen is compatible with follicular development, ovulation, and corpus luteum formation has been reviewed before and markedly reduced to even absent intrafollicular concentrations of estrogen are known to be compatible with follicular ''expansion,'' retrieval of fertilizable oocytes, and apparently normal embryo development (54). However, the rapid clearance of the aromatase inhibitors due to their relatively short half-life (around two days), the reversible nature of enzyme inhibition, and elevated levels of FSH, which induces new expression of aromatase enzyme, are factors that result in increasing estrogen production that has been demonstrated to be relatively normal at the time of ovulation; (21).

Outcome of Pregnancy Achieved after Treatment by Aromatase Inhibitors

Animal embryonic safety studies have found the aromatase inhibitor, anastrozole, to have no teratogenic or clastogenic effect, whereas there have been some concerns regarding teratogenic effects of letrozole if administered unintentionally during pregnancy (55). The short half-life of aromatase inhibitors, together with their administration in the early follicular phase, several days before ovulation, should result in their clearance before implantation takes place.

We reported the clinical outcome of early pregnancies achieved after the use of the aromatase inhibitor, letrozole, for ovulation induction or COH for IUI (14). In this nonrandomized cohort study, the outcome of pregnancies achieved after letrozole were compared along with the outcome of pregnancies achieved with other ovarian stimulation treatments with a control group of pregnancies spontaneously conceived without ovarian stimulation. In three tertiary referral centers over a two-year period, there were 394 pregnancy cycles in 345 infertile couples (133 pregnancies with 2.5 mg or 5 mg letrozole alone or with gonadotropins, 113 pregnancies with CC alone or with gonadotropins, 110 pregnancies with gonadotropins alone, and 38 pregnancies achieved without ovarian stimulation). Pregnancies conceived after IUI treatment were associated with comparable miscarriage and ectopic pregnancy rates compared to all other groups including the spontaneous conceptions. In addition, letrozole use was associated with a significantly lower rate of multiple gestation compared to CC consistent with the hypothesis of intact central estrogen negative-feedback mechanism on gonadotropin secretion.

A more recent multicenter study (56) that included 911 babies, 514 born after letrozole treatment, and 397 after CC treatment did not find any increase in the rates of major and minor malformations in babies conceived after letrozole treatment. Both groups (letrozole and clomiphene citrate) included infertility patients who received ovulation induction followed by IUI or timed intercourse. The study found seven newborns in the CC group (1.8 percent) and only one in the letrozole group (0.2 percent) to have congenital cardiac anomalies ($P = 0.02$). The incidence of cardiac anomalies in the letrozole group was slightly lower than the rate of congenital cardiac anomalies reported among all births (0.4–1.2 percent), and the CC rates were slightly higher. Ventricular septal defect was the predominant cardiac anomaly (five of eight newborns with cardiac anomalies), similar to the findings in spontaneously conceived pregnancies (57). These findings suggest that congenital cardiac anomalies are less frequent in the letrozole group than in the CC group and the general population.

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